

US EPA ARCHIVE DOCUMENT

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Aerobic and Anaerobic Transformation in Aquatic Sediment Systems

INTRODUCTION

1. Chemicals can enter shallow or deep surface waters by such routes as direct application, spray drift, run-off, drainage, waste disposal, industrial, domestic or agricultural effluent and atmospheric deposition. This Guideline describes a laboratory test method to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems. It is based on existing Guidelines (1)(2)(3)(4)(5)(6). An OECD Workshop on Soil/Sediment Selection, held in Belgirate, Italy in 1995 (7) agreed, in particular, on the number and type of sediments for use in this test. It also made recommendations relating to collection, handling and storage of sediment samples, based on the ISO Guidance (8). Such studies are required for chemicals which are directly applied to water or which are likely to reach the aqueous environment by the routes described above.

2. The conditions in natural aquatic sediment systems are often aerobic in the upper water phase. The surface layer of sediment can be either aerobic or anaerobic, whereas the deeper sediment is usually anaerobic. To encompass all of these possibilities both aerobic and anaerobic tests are described in this document. The aerobic test simulates an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient. The anaerobic test simulates a completely anaerobic water-sediment system. If circumstances indicate that it is necessary to deviate significantly from these recommendations, for example by using intact sediment cores or sediments that may have been exposed to the test substance, other methods are available for this purpose (9).

PRINCIPLE OF THE TEST

3. The method described in this guideline employs an aerobic and an anaerobic aquatic sediment system (see Annex 1) which allows:

- (i) the measurement of the transformation rate of the test substance in a water-sediment system,
- (ii) the measurement of the transformation rate of the test substance in the sediment,
- (iii) the measurement of the mineralisation rate of the test substance and/or its transformation products (when ¹⁴C-labelled test substance is used),
- (iv) the identification and quantification of transformation products in water and sediment phases including mass balance (when labelled test substance is used), and
- (v) the measurement of the distribution of the test substance and its transformation products between the two phases during a period of incubation in the dark (to avoid, for example, algal blooms) at constant temperature.

Half-lives, DT₅₀, DT₇₅ and DT₉₀ values are determined where the data warrant, but should not be extrapolated far past the experimental period (See Annex 2 for definitions).

4. At least two sediments and their associated waters are required for both the aerobic and the anaerobic studies respectively (7). However, there may be cases where more than two aquatic sediments should be used, for example, for a chemical that may be present in freshwater and/or marine environments.

APPLICABILITY OF THE TEST

5. The method is generally applicable to chemical substances (unlabelled or labelled) for which an analytical method with sufficient accuracy and sensitivity is available. It is applicable to slightly volatile, non-volatile, water-soluble or poorly water-soluble compounds. The test should not be applied to chemicals which are highly volatile from water (e.g. fumigants, organic solvents) and thus cannot be kept in water and/or sediment under the experimental conditions of this test.

6. The method has been applied so far to study the transformation of chemicals in fresh waters and sediments, but in principle can also be applied to estuarine/marine systems. It is not suitable to simulate conditions in flowing water (e.g. rivers) or the open sea.

INFORMATION ON THE TEST SUBSTANCE

7. Non-labelled or isotope-labelled test substance can be used to measure the rate of transformation although labelled material is preferred. Labelled material is required for studying the pathway of transformation and for establishing a mass balance. ¹⁴C-labelling is recommended, but the use of other isotopes, such as ¹³C, ¹⁵N, ³H, ³²P, may also be useful. As far as possible, the label should be positioned in the most stable part(s) of the molecule¹. The chemical and/or radiochemical purity of the test substance should be at least 95%.

8. Before carrying out a test, the following information about the test substance should be available:

- (a) solubility in water [OECD Guideline 105] (10);
- (b) solubility in organic solvents;
- (c) vapour pressure [OECD Guideline 104] (10) and Henry's Law constant;
- (d) n-octanol/water partition coefficient [OECD Guidelines 107 and 117] (10);
- (e) adsorption coefficient (K_d , K_f or K_{oc} , where appropriate) [OECD Guideline 106] (10);
- (f) hydrolysis [OECD Guideline 111] (10);
- (g) dissociation constant (pK_a) [OECD Guideline 112] (10).
- (h) chemical structure of the test substance and position of the isotope-label(s), if applicable.

Note: The temperature at which these measurements were made should be reported.

9. Other useful information may include data on toxicity of the test substance to microorganisms, data on ready and/or inherent biodegradability, and data on aerobic and anaerobic transformation in soil.

10. Analytical methods (including extraction and clean-up methods) for identification and quantification of the test substance and its transformation products in water and in sediment should be available (see also paragraphs 14 and 15).

¹ For example, if the substance contains one ring, labelling on this ring is required; if the test substance contains two or more rings, separate studies may be needed to evaluate the fate of each labelled ring and to obtain suitable information on formation of transformation products.

REFERENCE SUBSTANCES

11. Reference substances should be used for the identification and quantification of transformation products by spectroscopic and chromatographic methods.

DEFINITIONS AND UNITS

12. See Annex 2.

QUALITY CRITERIA**Recovery**

13. Extraction and analysis of, at least, duplicate water and sediment samples immediately after the addition of the test substance give a first indication of the repeatability of the analytical method and of the uniformity of the application procedure for the test substance. Recoveries for later stages of the experiments are given by the respective mass balances (when labelled material is used). Recoveries should range from 90% to 110% for labelled chemicals (6) and from 70% to 110% for non-labelled chemicals.

Repeatability and sensitivity of analytical method

14. Repeatability of the analytical method (excluding the initial extraction efficiency) to quantify test substance and transformation products can be checked by duplicate analysis of the same extract of the water or the sediment samples which were incubated long enough for formation of transformation products.

15. The limit of detection (LOD) of the analytical method for the test substance and for the transformation products should be at least $0.01 \text{ mg}\cdot\text{kg}^{-1}$ in water or sediment (as test substance) or 1% of the initial amount applied to a test system whichever is lower. The limit of quantification (LOQ) should also be specified.

Accuracy of transformation data

16. Regression analysis of the concentrations of the test substance as a function of time gives the appropriate information on the accuracy of the transformation curve and allows the calculation of the confidence limits for half-lives (if pseudo first-order kinetics apply) or DT_{50} values and, if appropriate, DT_{75} and DT_{90} values.

DESCRIPTION OF THE METHOD**Test system and apparatus**

17. The study should be performed in glass containers (e.g. bottles, centrifuge tubes), unless preliminary information (such as n-octanol-water partition coefficient, sorption data, etc.) indicates that the test substance may adhere to glass, in which case an alternative material (such as Teflon) may have to be

considered. Where the test substance is known to adhere to glass, it may be possible to alleviate this problem using one or more of the following methods:

- determine the mass of test substance and transformation products sorbed to glass;
- ensure a solvent wash of all glassware at the end of the test;
- use of formulated products (see also paragraph 36);
- use an increased amount of co-solvent for addition of test substance to the system; if a co-solvent is used it should be a co-solvent that does not solvolyse the test substance.

18. Examples of typical test apparatus, i.e. gas flow-through and biometer-type systems, are shown in Annexes 3 and 4, respectively (11). Other useful incubation systems are described in reference 12. The design of the experimental apparatus should permit the exchange of air or nitrogen and the trapping of volatile products. The dimensions of the apparatus must be such that the requirements of the test are complied with (see paragraph 32). Ventilation may be provided by either gentle bubbling or by passing air or nitrogen over the water surface. In the latter case gentle stirring of the water from above may be advisable for better distribution of the oxygen or nitrogen in the water. CO₂-free air should not be used as this can result in increases in the pH of the water. In either case, disturbance of the sediment is undesirable and should be avoided as far as possible. Slightly volatile chemicals should be tested in a biometer-type system with gentle stirring of the water surface. Closed vessels with a headspace of either atmospheric air or nitrogen and internal vials for the trapping of volatile products can also be used (13). Regular exchange of the headspace gas is required in the aerobic test in order to compensate for the oxygen consumption by the biomass.

19. Suitable traps for collecting volatile transformation products include but are not restricted to 1 mol·dm⁻³ solutions of potassium hydroxide or sodium hydroxide for carbon dioxide² and ethylene glycol, ethanolamine or 2% paraffin in xylene for organic compounds. Volatiles formed under anaerobic conditions, such as methane, can be collected, for example, by molecular sieves. Such volatiles can be combusted, for example, to CO₂ by passing the gas through a quartz tube filled with CuO at a temperature of 900 °C and trapping the CO₂ formed in an absorber with alkali (14).

20. Laboratory instrumentation for chemical analysis of test substance and transformation products is required (e.g. gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), thin-layer chromatography (TLC), mass spectroscopy (MS), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), etc.), including detection systems for radiolabelled or non-labelled chemicals as appropriate. When radiolabelled material is used a liquid scintillation counter and combustion oxidiser (for the combustion of sediment samples prior to analysis of radioactivity) will also be required.

21. Other standard laboratory equipment for physical-chemical and biological determinations (see Table 1, paragraph 26), glassware, chemicals and reagents are required as appropriate.

Selection and number of aquatic sediments

22. The sampling sites should be selected in accordance with the purpose of the test in any given situation. In selecting sampling sites, the history of possible agricultural, industrial or domestic inputs to the catchment and the waters upstream must be considered. Sediments should not be used if they have been contaminated with the test substance or its structural analogues within the previous 4 years.

² As these alkaline absorption solutions also absorb the carbon dioxide from the ventilation air and that formed by respiration in aerobic experiments, they have to be exchanged at regular intervals to avoid their saturation and thus loss of their absorption capacity.

Sediment selection

23. Two sediments are normally used for the aerobic studies (7). The two sediments selected should differ with respect to organic carbon content and texture. One sediment should have a high organic carbon content (2.5-7.5%) and a fine texture, the other sediment should have a low organic carbon content (0.5-2.5%) and a coarse texture. The difference between the organic carbon contents should normally be at least 2%. "Fine texture" is defined as a [clay + silt]³ content of >50% and "coarse texture" is defined as a [clay + silt] content of <50%. The difference in [clay + silt] content for the two sediments should normally be at least 20%. In cases, where a chemical may also reach marine waters, at least one of the water-sediment systems should be of marine origin.

24. For the strictly anaerobic study, two sediments (including their associated waters) should be sampled from the anaerobic zones of surface water bodies (7). Both the sediment and the water phases should be handled and transported carefully under exclusion of oxygen.

25. Other parameters may be important in the selection of sediments and should be considered on a case-by-case basis. For example, the pH range of sediments would be important for testing chemicals for which transformation and/or sorption may be pH-dependent. pH-dependency of sorption might be reflected by the pK_a of the test substance.

Characterisation of water-sediment samples

26. Key parameters that must be measured and reported (with reference to the method used) for both water and sediment, and the stage of the test at which those parameters are to be determined are summarised in the Table hereafter. For information, methods for determination of these parameters are given in references (15)(16)(17)(18).

27. In addition, other parameters may need to be measured and reported on a case by case basis e.g. for freshwater: particles, alkalinity, hardness, conductivity, NO₃/PO₄ (ratio and individual values); for sediments: cation exchange capacity, water holding capacity, carbonate, total nitrogen and phosphorus; and for marine systems: salinity). Analysis of sediments and water for nitrate, sulfate, bioavailable iron, and possibly other electron acceptors may be also useful in assessing redox conditions, especially in relation to anaerobic transformation.

³ [Clay + silt] is the mineral fraction of the sediment with particle size of < 50 µm.

Measurement of parameters for characterisation of water-sediment samples (7)(19)(20)

Parameter	Stage of test procedure					
	field sampling	post-handling	start of acclimation	start of test	during test	end of test
Water						
Origin/source	x					
Temperature	x					
pH	x		x	x	x	x
TOC			x	x		x
O ₂ concentration*	x		x	x	x	x
Redox potential*			x	x	x	x
Sediment						
Origin/source	x					
Depth of layer	x					
pH		x	x	x	x	x
Particle size distribution		x				
TOC		x	x	x		x
Microbial biomass**		x		x		x
Redox potential *	Observation (colour/smell)		x	x	x	x

* Recent research results have shown that measurements of water oxygen concentrations and of redox potentials have neither a mechanistic nor a predictive value as far as growth and development of microbial populations in surface waters are concerned (21)(22). Determination of the biochemical oxygen demand (BOD, at field sampling, start and end of test) and of concentrations of micro/macro nutrients Ca, Mg and Mn (at start and end of test) in water and the measurement of total N and total P in sediments (at field sampling and end of test) may be better tools to interpret and evaluate aerobic biotransformation rates and routes.

** Microbial respiration rate method (23), fumigation method (24) or plate count measurements (e.g. bacteria, actinomycetes, fungi and total colonies) for aerobic studies; methanogenesis rate for anaerobic studies.

Collection, handling and storage**Collection**

28. The draft ISO guidance on sampling of bottom sediment (8) should be used for sampling of sediment. Sediment samples should be taken from the entire 5 to 10 cm upper layer of the sediment. Associated water should be collected from the same site or location and at the same time as the sediment. For the anaerobic study, sediment and associated water should be sampled and transported under exclusion of oxygen (25) (see also paragraph 24). Some sampling devices are described in the literature (8)(20).

Handling

29. The sediment is separated from the water by filtration and the sediment wet-sieved to a 2 mm-sieve using excess location water that is then discarded. Then known amounts of sediments and water are mixed at the desired ratio (see paragraph 32) in incubation flasks and prepared for the acclimation period (see paragraph 31). For the anaerobic study, all handling steps have to be done under exclusion of oxygen (26)(27)(28)(29)(30).

Storage

30. Use of freshly sampled sediment and water is strongly recommended, but if storage is necessary, sediment and water should be sieved as described above and stored together, water-logged (6-10 cm water layer), in the dark, at $4 \pm 2^{\circ}\text{C}$ ⁴ for a maximum of 4 weeks (7)(8)(20). Samples to be used for aerobic studies should be stored with free access of air (e.g. in open containers), whereas those for anaerobic studies under exclusion of oxygen. Freezing of sediment and water and drying-out of the sediment must not occur during transportation and storage.

Preparation of the sediment/water samples for the test

31. A period of acclimation should take place prior to adding the test substance, with each sediment/water sample being placed in the incubation vessel to be used in the main test, and the acclimation to be carried out under exactly the same conditions as the test incubation (see paragraphs 32 and 33). The acclimation period is the time needed to reach reasonable stability of the system, as reflected by pH, oxygen concentration in water, redox potential of the sediment and water, and macroscopic separation of phases. The period of acclimation should normally last between one week and two weeks and should not exceed four weeks. Results of determinations performed during this period should be reported.

PERFORMANCE OF THE TEST**Test conditions**

32. The test should be performed in the incubation apparatus (see paragraphs 17-19) with a water:sediment volume ratio between 3:1 and 4:1, and a sediment layer of 2.5 cm (± 0.5 cm). A minimum amount of 50 g of sediment (dry weight basis) per incubation vessel is recommended.

⁴ Recent studies have shown that storage at 4°C can lead to a decrease of the organic carbon content of the sediment which may possibly result in a decrease of microbial activity (31).

33. The test should be performed in the dark at a constant temperature in the range of 10 to 30 °C. A temperature of $(20 \pm 2)^{\circ}\text{C}$ is appropriate. Where appropriate, an additional lower temperature (e.g. 10 °C) may be considered on a case-by-case basis, depending on the information required from the test. Incubation temperature should be monitored and reported.

Treatment and application of test substance

34. One test concentration of chemical is used⁵. For crop protection chemicals applied directly to water bodies, the maximum dosage on the label should be taken as the maximum application rate calculated on the basis of the surface area of the water in the test vessel. In all other cases, the concentration to be used should be based on predictions from environmental emissions. Care must be taken to ensure that an adequate concentration of test substance is applied in order to characterise the route of transformation and the formation and decline of transformation products. It may be necessary to apply higher doses (e.g. 10 times) in situations where test substance concentrations are close to limits of detection at the start of the study and/or where major transformation products could not readily be detected when present at 10% of the test substance application rate. However, if higher test concentrations are used they should not have a significant adverse effect on the microbial activity of the water-sediment system. In order to achieve a constant concentration of test substance in vessels of differing dimensions an adjustment to the quantity of the material applied may be considered appropriate, based on the depth of the water column in the vessel in relation to the depth of water in the field (which is assumed to be 100 cm, but other depths can be used). See Annex 5 for an example calculation.

35. Ideally the test substance should be applied as an aqueous solution into the water phase of the test system. If unavoidable, the use of low amounts of water miscible solvents (such as acetone, ethanol) is permitted for application and distribution of the test substance, but this should not exceed 1% v/v and should not have adverse effects on microbial activity of the test system. Care should be exercised in generating the aqueous solution of the test substance - use of generator columns and pre-mixing may be appropriate to ensure complete homogeneity. Following addition of the aqueous solution to the test system, gentle mixing of the water phase is recommended, disturbing the sediment as little as possible.

36. The use of formulated products is not routinely recommended as the formulation ingredients may affect the distribution of the test substance and/or transformation products between water and sediment phases. However, for poorly water-soluble test substances, the use of formulated material may be an appropriate alternative.

37. The number of incubation vessels depends on the number of sampling times (see paragraph 38). A sufficient number of test systems should be included so that two systems may be sacrificed at each sampling time. Where control units of each aquatic sediment system are employed, they should not be treated with the test substance. The control units can be used to determine the microbial biomass of the sediment and the total organic carbon of the water and sediment at the termination of the study. Two of the control units (i.e. one control unit of each aquatic sediment) can be used to monitor the required parameters in the sediment and water during the acclimation period (see Table in paragraph 26). Two additional control units have to be included if the test substance is applied by means of a solvent to measure adverse effects on the microbial activity of the test system.

⁵ Test with a second concentration can be useful for chemicals that reach surface waters by different entry routes resulting in significantly different concentrations, as long as the lower concentration can be analysed with sufficient accuracy.

Test duration and sampling

38. The duration of the experiment should normally not exceed 100 days (6), and should continue until the degradation pathway and water/sediment distribution pattern are established or when 90 % of the test substance has been removed by transformation and/or volatilisation. The number of sampling times should be at least six (including zero time), with an optional preliminary study (see paragraph 40) being used to establish an appropriate sampling regime and the duration of the test, unless sufficient data are available on the test substance from previous studies. For hydrophobic test substances, additional sampling points during the initial period of the study may be necessary in order to determine the rate of distribution between water and sediment phases.

39. At appropriate sampling times, whole incubation vessels (in replicate) are removed for analysis. Sediment and overlying water are analysed separately⁶. The surface water should be carefully removed with minimum disturbance of the sediment. The extraction and characterisation of the test substance and transformation products should follow appropriate analytical procedures. Care should be taken to remove material that may have adsorbed to the incubation vessel or to interconnecting tubing used to trap volatiles.

Optional preliminary test

40. If duration and sampling regime cannot be estimated from other relevant studies on the test substance, an optional preliminary test may be considered appropriate, which should be performed using the same test conditions proposed for the definitive study. Relevant experimental conditions and results from the preliminary test, if performed, should be briefly reported.

Measurements and analysis

41. Concentration of the test substance and the transformation products at every sampling time in water and sediment should be measured and reported (as a concentration and as percentage of applied). In general, transformation products detected at $\geq 10\%$ of the applied radioactivity in the total water-sediment system at any sampling time should be identified unless reasonably justified otherwise. Transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limits given above, as this may indicate persistence. The latter should be considered on a case by case basis, with justifications being provided in the report.

42. Results from gases/volatiles trapping systems (CO₂ and others, i.e. volatile organic compounds) should be reported at each sampling time. Mineralisation rates should be reported. Non-extractable (bound) residues in sediment are to be reported at each sampling point.

DATA AND REPORTING

Treatment of results and calculations

43. Total mass balance or recovery (see paragraph 13) of added radioactivity is to be calculated at every sampling time. Results should be reported as a percentage of added radioactivity. Distribution of radioactivity between water and sediment should be reported as concentrations and percentages, at every sampling time.

⁶ In cases where rapid re-oxidation of anaerobic transformation products may readily occur, anaerobic conditions should be maintained during sampling and analysis.

44. Half-life, DT_{50} and, if appropriate, DT_{75} and DT_{90} of the test substance should be calculated along with their confidence limits (see paragraph 16). Information on the rate of dissipation of the test substance in the water and sediment can be obtained through the use of appropriate evaluation tools. These can range from application of pseudo-first order kinetics, empirical curve-fitting techniques which apply graphical or numerical solutions and more complex assessments using, for example, single- or multi-compartment models. Further details can be obtained from the relevant published literature (32)(33)(34).

45. All approaches have their strengths and weaknesses and vary considerably in complexity. An assumption of first-order kinetics may be an oversimplification of the degradation and distribution processes, but when possible gives a term (the rate constant or half-life) which is easily understood and of value in simulation modelling and calculations of predicted environmental concentrations. Empirical approaches or linear transformations can result in better fits of curves to data and therefore allow better estimation of half-lives, DT_{50} and, if appropriate, DT_{75} and DT_{90} values. The use of the derived constants, however, is limited. Compartment models can generate a number of useful constants of value in risk assessment that describe the rate of degradation in different compartments and the distribution of the chemical. They should also be used for estimation of rate constants for the formation and degradation of major transformation products. In all cases, the method chosen must be justified and the experimenter should demonstrate graphically and/or statistically the goodness of fit.

Test report

46. The report must include the following information:

Test substance:

- common name, chemical name, CAS number, structural formula (indicating position of the label(s) when radiolabelled material is used) and relevant physical-chemical properties;
- purity (impurities) of test substance;
- radiochemical purity of labelled chemical and molar activity (where appropriate).

Reference substances:

- chemical name and structure of reference substances used for the characterisation and/or identification of transformation products.

Test sediments and waters:

- location and description of aquatic sediment sampling site(s) including, if possible, contamination history;
- all information relating to the collection, storage (if any) and acclimation of water-sediment systems;
- characteristics of the water-sediment samples as listed in Table in paragraph 26.

Test conditions:

- test system used (flow-through, biometer, way of ventilation, method of stirring, water volume, mass of sediment, thickness of both water and sediment layer, dimension of test vessels, etc.)
- application of test substance to test system: test concentration used, number of replicates and controls mode of application of test substance (e.g. use of solvent if any), etc.

- incubation temperature;
- sampling times;
- extraction methods and efficiencies as well as analytical methods and detection limits;
- methods for characterisation/identification of transformation products;
- deviations from the test protocol or test conditions during the study.

Results:

- raw data figures of representative analyses (all raw data have to be stored in the GLP-archive);
- repeatability and sensitivity of the analytical methods used;
- rates of recovery (% values for a valid study are given in paragraph 13);
- tables of results expressed as % of the applied dose and in $\text{mg}\cdot\text{kg}^{-1}$ in water, sediment and total system (% only) for the test substance and, if appropriate, for transformation products and non-extractable radioactivity;
- mass balance during and at the end of the studies;
- a graphical representation of the transformation in the water and sediment fractions and in total system (including mineralisation);
- mineralisation rates;
- half-life, DT_{50} and, if appropriate, DT_{75} and DT_{90} values for the test substance and, where appropriate, for transformation products including confidence limits in water, sediment and in total system;
- an assessment of the transformation kinetics of the test substance and, where appropriate, the transformation products;
- a proposed pathway of transformation, where appropriate;
- discussion of results.

LITERATURE

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ANNEX 1**GUIDANCE ON THE AEROBIC AND THE ANAEROBIC TEST SYSTEMS****Aerobic test system**

The aerobic test system described in this test guideline consists of an aerobic water layer (typical oxygen concentrations range from 7 to 10 mg·L⁻¹) and a sediment layer, aerobic at the surface and anaerobic below the surface (typical average redox potentials (E_h) in the anaerobic zone of the sediment range from -80 to -190 mV). Moistened air is passed over the surface of the water in each incubation unit to maintain sufficient oxygen in the head space.

Anaerobic test system

For the anaerobic test system, the test procedure is essentially the same as that outlined for the aerobic system with the exception that moistened nitrogen is passed above the surface of the water in each incubation unit to maintain a head space of nitrogen. The sediment and water are regarded as anaerobic once the redox potential (E_h) is lower than -100 mV.

In the anaerobic test, assessment of mineralisation includes measurement of evolved carbon dioxide and methane.

ANNEX 2DEFINITIONS AND UNITS

Standard International (SI) units should be used in any case.

Test substance: any substance, whether the parent or relevant transformation products.

Transformation products: all substances resulting from biotic and abiotic transformation reactions of the test substance including CO₂ and bound residues.

Bound residues: “Bound residues” represent compounds in soil, plant or animal that persist in the matrix in the form of the parent substance or its metabolite(s) after extractions. The extraction method must not substantially change the compounds themselves or the structure of the matrix.

The nature of the bond can be clarified in part by matrix-altering extraction methods and sophisticated analytical techniques. To date, for example, covalent ionic and sorptive bonds, as well as entrapments, have been identified in this way. In general, the formation of bound residues reduces the bioaccessibility and the bioavailability significantly (1) [modified from IUPAC 1984 (2)].

Aerobic transformation (oxidising): reactions occurring in the presence of molecular oxygen (3).

Anaerobic transformation (reducing): reactions occurring under exclusion of molecular oxygen (3).

Natural waters are surface waters obtained from ponds, rivers, streams, etc.

Sediment is a mixture of mineral and organic chemical constituents, the latter containing compounds of high carbon and nitrogen content and of high molecular masses. It is deposited by natural water and forms an interface with that water.

Mineralisation is the complete degradation of an organic compound to CO₂, H₂O under aerobic conditions, and CH₄, CO₂ and H₂O under anaerobic conditions. In the context of this guideline, when radiolabelled compound is used, mineralisation means extensive degradation of a molecule during which a labelled carbon atom is oxidised or reduced quantitatively with release of the appropriate amount of ¹⁴CO₂ or ¹⁴CH₄, respectively.

- (1) DFG: Pesticide Bound Residues in Soil. Wiley-VCH (1998).
- (2) T.R. Roberts: Non-extractable pesticide residues in soils and plants. Pure Appl. Chem. 56, 945-956 (IUPAC 1984).
- (3) OECD (1981). Test Guideline 304A: Inherent Biodegradability in Soil.

ANNEX 2 (continued)

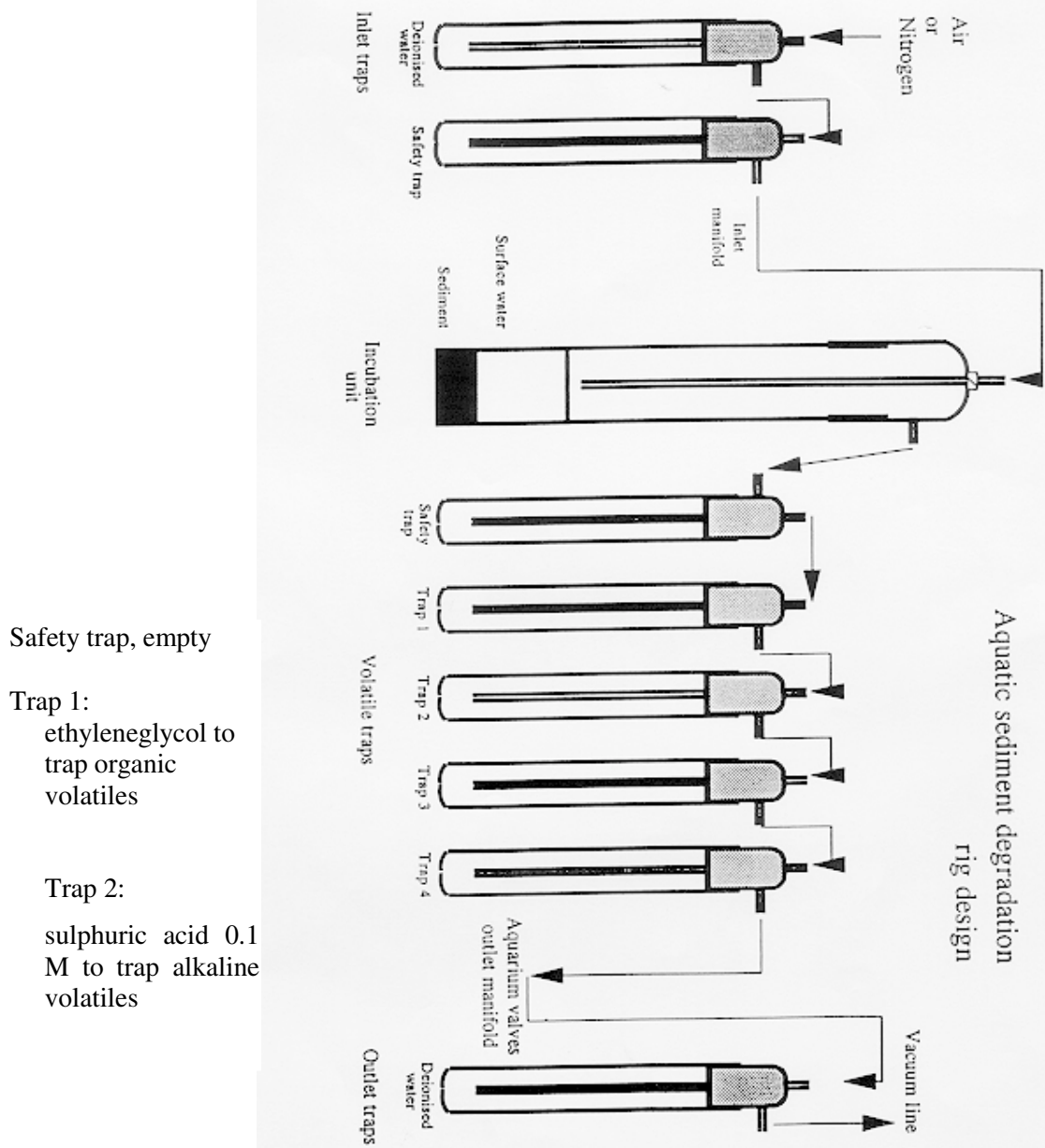
Half-life, $t_{0.5}$, is the time taken for 50% transformation of a test substance when the transformation can be described by first-order kinetics; it is independent of the initial concentration.

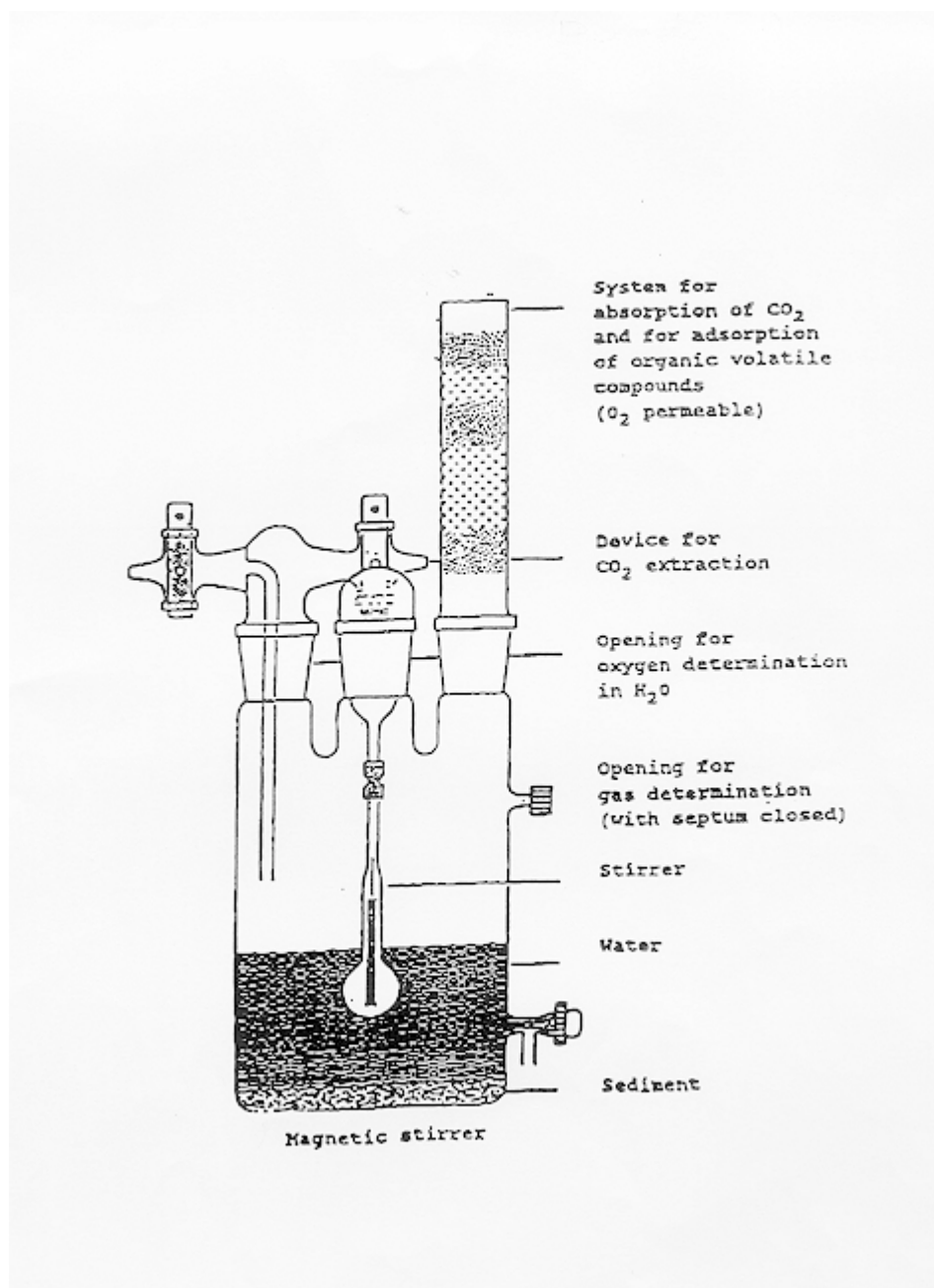
DT₅₀ (Disappearance Time 50) is the time within which the initial concentration of the test substance is reduced by 50%.

DT₇₅ (Disappearance Time 75) is the time within which the initial concentration of the test substance is reduced by 75%.

DT₉₀ (Disappearance Time 90) is the time within which the initial concentration of the test substance is reduced by 90%.

ANNEX 3
EXAMPLE OF A GAS FLOW-THROUGH APPARATUS



ANNEX 4EXAMPLE OF A BIOMETER APPARATUS

ANNEX 5EXAMPLE CALCULATION FOR APPLICATION DOSE TO TEST VESSELS

Cylinder internal diameter:	= 8 cm
Water column depth not including sediment:	= 12 cm
Surface area: 3.142×4^2	= 50.3 cm^2
Application rate: 500 g test substance/ha corresponds to $5 \text{ } \mu\text{g}/\text{cm}^2$	
Total μg : 5×50.3	= 251.5 μg
Adjust quantity in relation to a depth of 100 cm:	
$12 \times 251.5 \div 100$	= 30.18 μg
Volume of water column: 50.3×12	= 603 mL
Concentration in water: $30.18 \div 603$	= 0.050 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{L}$